REMARKS

Before this amendment claims 1-19 and 23-38 were pending. Claims 14-19 and 27-31 have been withdrawn from consideration by the Examiner and the remaining claims stand rejected. In response, claim 5 is cancelled without prejudice or disclaimer, and claims 1, 24, 32, 34-35, and 37 are amended. Amendments are provided for improved clarity and do not add new terms, being supported by the specification as filed. Reconsideration and allowance of examined claims 1-4, 6-19 and 23-38 are requested.

Specification

The Examiner has objected to the specification for various informalities. In response, applicants have amended the specification as suggested by the Examiner. Reconsideration and removal of the informality objections are solicited.

Claim Rejections - 35 USC § 112, first paragraph

On page 4 of the Office Action the Examiner has rejected claims 1-13, 23-26 and 32-38 on alleged 35 U.S.C. 112, first paragraph enablement grounds. For the purpose of advancing prosecution applicants have merged claim 5 with claim 1 by adding the recitation "the hyaluronic acid derivative is a hyaluronic acid ester" to claim 1.

Claim Rejections - 35 USC § 112, second paragraph

On page 5 of the Office Action the Examiner has rejected claims 24 and 32-38 under 35 U.S.C. 112, second paragraph, on alleged indefinite grounds. Applicants have amended claim 24 as suggested by the Examiner. Applicants have amended claim 32 by deleting "optionally" and have amended claims 34 and 35 by removing the reference to "type" from these claims. Claims 34 and 35 have been further amended to present "connective and supportive apparatus" as new clarifying feature element of these dependent claims.

Reconsideration and allowance are respectfully requested.

On the bottom of page 5, the Examiner argues that "[t]he structure of an apparatus that is connective and supportive is uncertain." In response, applicants point out that skilled artisans in the field of (biocompatible and biodegradable

composite matrixes for body implants), connective tissue and supportive tissue, which comprises collagen complexes, elastin complexes, muscle complexes and the like are routinely considered. The "apparatus" within the body that supports an introduced object, such as that claimed, generally is of high importance and known to comprise such structures, alone or in combination. The term "connective" is well known to mean that of connecting hard objects (such as bones) to other structures such as tendons. Accordingly, applicants respectively request reconsideration and removal of this rejection.

On the top of page 6 of the Office Action, the Examiner has argued that "hyaluronic acid is a glycosaminoglycan" and has suggested that the term "further" be deleted. Applicants point out that "biologically active" glycosaminoglycans in particular are intended and that hyaluronic acid per se, is omitted by the original use of the term "further glycosaminoglycans." Here, the term further means "additional, not previously stated." Claim 37 now has been amended to make this point more clear. Reconsideration and allowance are requested.

Claim Rejections - 35 USC § 103(a)

On pages 6-9 of the Office Action, the Examiner has rejected claims 1-4, 11-13, 23-25, 32-36 and 38, as obvious over over Liu et al (5,866,165), and "if necessary in view of Aoyagi et al (6,423,347) and Bishop et al (5,834,232)."

Applicants point out that the material described in the references are formed differently and have different properties than those contemplated. Moreover, a skilled artisan in this field reading the claims would know that the claims refer to the types of starting materials with differing structures and properties from that described in the references. Accordingly, taken separately or in combination the references do not obviate the claimed invention.

As an example of a claimed structural difference, the **Liu et al., 1998**, patent describes a composite matrix with a hyaluronan derivative and collagen, but in comparison to the present specification uses distinct reagents, a completely different manufacturing process and consecutively a completely different endproduct.

Liu et al. and applicants both use hyaluronic acid as a basic material. But the modifying procedure of use differs completely and results in completely

different hyaluronan derivatives. Liu activates the hyaluronic acid in their procedure. The hyaluronic acid is modified by opening the sugar rings and oxidizing the hydroxyls to aldehydes. In contrast the present invention avoids this by, for example derivatizing hyaluronan in order to increase chemical stability against enzymatic digestion by an increase in hydrophobic character. Applicants therefore use a hyaluronbenzylester.

Another structural difference, that a skilled artisan would apprehend upon a reading of the claims and attached specification is the use of hydrolysed collagen for the composite matrix. This material is a component mixture of two reagents that generally lacks covalent crosslinks between the components. Liu et al. in contrast use extended covalent bridging between their hyaluronan and collagen components.

Liu et al. never described hydrolysed forms of collagen for their material. This fact is very important for the claimed composite matrix, because only with hydrolysed collagen can both reagents simultaneously be solvated in one solvent (HFIP). This, although not literally recited as such, nevertheless is part of the claims as being inherent in the operation of the method and in the structure of the materials. That is, a skilled reader will understand that only hydrolysed forms of collagen will work as claimed and understands this difference.

Because of this major structural and functional difference from the cited art, reconsideration and allowance are requested.

A further difference is that Liu produces mainly non porous materials, e.g. gel in combination with fibrin, in contrast to applicant's highly porous matrix. To the extent Liu describes porous materials, Liu achieves this by freeze drying in contrast to applicant's air drying procedure. Finally, Liu never control the pore size of their biomaterial, which fundamentally is possible with the claimed manufacturing procedure. The pore size control is important for best results in a biological system.

Applicants further note that Liu et al claim a biomaterial that can be seeded with cells on the scaffold but never inside the scaffold (page 11, line 10ff). This is in contrast to the claimed biomaterial and is an inherent feature of the material. Furthermroe, if an ingrowth of cells should be achieved, Liu uses another

scaffold that is covered by a film of their construct. This is in contrast to applicant's biomaterial as well.

Reconsideration and allowance are further requested.

Bishop et al. produce protein gels with transglutaminase induced crosslinks. Bishop's biomaterial is not porous, is not a composite material, but solely consists of collagen and generally lacks hyaluronan, and thus differs greatly from the claimed invention.

Aoyagi et al. describe a fish collagen product that can be used as a drug for treatment of rheumatoid arthritis. This is completely different from applicant's biomaterial setup. Aoyagi describe the enteral administration of notochord obtained from different species of sturgen (column 7). Moreover, the Aoyagi patent was filed on October 3, 2000, which is later than the priority date of the present application. Aoyagi does not apply for this reason as well.

Valentini et al. describe a matrix consisting of derivatized hyaluronic acid that was manufactured by applicants according to PCT-Patent WO 97/45532. This starting material has positive properties regarding delayed degradation, biocompatibility and form stability by handling. A disadvantage was the reduced adherence of mesenchymal cells on this estrified hyaluronic acid (Hyaff 11, abbreviated as HA in figure 5) as shown in figure 5. Using common sterilization methods (gamma-irradiation) a further decrease of cell adherence on the 100% HA matrix could be detected (figure 5). By mixing of hydrolysed collagen with HA the cell adhesion on applicant's matrix could be significantly improved. The related uptake of cells represents an important factor in the manufacturing of cell-matrix-implants and in the consequence in the engineering of cartilage.

This idea of improvement of cell adhesion by collagen has not been revealed by Valentini et al. These authors focus their interest on products without collagen. In certain circumstances they use a collagen layer only as a coating of the hyaluronic acid matrix. This layer therefore represents a carrier for further additives. Valentini et al. do not use hydrolyzed collagen.

Applicants emphasize that in contrast to fibrillar collagen, only the hydrolysed form of collagen dissolves in HFIP and yields a mixed solution with HA as a source material for the final composite scaffold product as claimed. After addition of 1% up to 40% of hydrolysed collagen a clearly improved cell adherence can be achieved. The sterilization by gamma irridiation does not diminish the cell adherence to applicant's composite matrix, which is very important for the application of applicant's scaffold *in vivo* (figure 5).

In comparison to matrices made of 100% hyaluronic acid ester alone a markedly increased cell proliferation of mesenchymal stem cells can be obtained by use of applicant's composite matrix (figure 6). The stimulation of proliferation by hydrolysed collagen requires stabilization of the matrix product by mixing with HA. Therefore a ratio of HA-ester : hydrolyzed collagen of 99:1 to 60:40 is recommendable. An also improved cell differentiation of mesenchymal stem cells on their way to osteochondral tissue can be achieved with a ratio of hyaluronic acid ester : hydrolysed collagen of 70:30. This could be shown in *in vitro* and *in vivo* test series.

To compare the manufacturing process of Valentini et al. with the claimed method, applicants manufactured matrices by using both techniques and examined their structure by scanning electron microscopy. The results are presented in figure 7 ABC and show that the matrices are different products.

Besides the addition of hydrolysed collagen the claimed invention also differs from WO 97/45532 in respect to the manufacturing process of the three dimensional scaffold resulting in a dual porous structure of the scaffold. In addition to the main pores which are formed by the porogen (salt) a second class of macro pores are achieved (figure 7). These extra pores result from an additional manufacturing step, during which the solvent 1 (HFIP) is removed by drying. The solution of HA and hydrolysed collagen between the grains of porogen shrinks, leading to the formation of a second class of pores between the main pores by air influx. This three dimensional scaffold shows a high degree of interconnectivity between the primary and secondary pores. Therefore compared with the single-pore structure of the WO

97/45532 matrix, applicants scaffold offers by its double pore structure superior space conditions for the seeding with cells.

WO 97/45532 shows a ratio of porogen to hyaluronic acid ester of 9:1 resp. 15:1. This means, that there is a relative high amount of HA in the matrix walls. They appear to be very thick and solid without an additional secondary macro porosity. However higher magnifications showed micro pores which cannot contribute to cell seeding because of their small size (Fig. 7 A,B,C).

The claimed invention in contrast uses a ratio of porogen to HA between 40:1 and 60:1. By this means applicant markedly reduced the amount of solid components in the scaffold without dimunition of its biomechanical stability. This high stability results from the thin but uniform and compact structure of the pore walls which can be seen at high magnifications (Fig.7C).

For these further reasons reconsideration and allowance are requested.

(See appended figures)

See figure 7: Pictures A, B, C show scanning electron microscope pictures of the artificial extracellular matrix (Magnifications: 100x, 500x und 3000x) manufactured according to the manufacturing process presented in the specification, in contrast to the matrix manufactured according to the process of Valentini ("D2" PCT-Patent WO 97/45532). The pictures give evidence that the methodic differences in both manufacturing techniques result in two different products. In addition to the different biological properties mentioned before, they show significant structural differences at microscopic and ultramicroscopic levels.

The formula of Valentini does not suppose any **addition of gelatin**, therefore both matrices in this comparison are composed of merely hyaluronic acid ester. It should be emphasised that addition of gelatin in concentrations applicant's propose

has no detectable influence on the structure of the scaffold, but it improves considerably the biological properties of the scaffold.

The **concentration of hyaluronic acid ester** in this comparison is in accordance to applicant's presented manufacturing process. A several times higher concentration as recommended by Valentini would lead to a much stronger negative impact on the pore creation than that which can already be shown in his matrix product.

The comparison shows primarily two methodically caused differences in structure of the both matrix products. On the one hand it is the formation of secondary pores besides the main pores of the matrix (in our product – intense, in the matrix of Valentini – negligible), on the other hand the different structure of the pore walls (in our product – thin and very compact walls, in the matrix of Valentini– voluminous and foam like).

Secondary pores occur in the areas of the matrix which are not occupied with main pores (between the porogen grains) as the extraction of the first solvent is accomplished by air drying. During this step the first solvent evaporates from the solution between the grains of the porogen and is replaced bit by bit by air. Thereby a network of interconnective secondary pores develops in addition to the main pores which are subsequent washed out in our method by elution of the porogen with the second solvent. In the manufacturing process of Valentini the first solvent is extracted directly from the mixture of the hyaluronic acid ester solution and the porogen without drying by use of the second solvent in which the hyaluronic acid ester is not solvable. Because of this solvent substitution the hyaluronic acid ester merges into a dispersion state and subsequently falls out very fine and foam-like, resulting in a nearly complete filling of the pore interstices. Therefore no secondary pores can develop.

The structure of the pore walls in applicant's matrix product takes shape by the drying step. The drying solution of the hyaluronic acid ester spreads at the walls of the crystalls of the porogen and condenses to very thin, homogenous, compact

layers. In the manufacturing process of Valentini the hyaluronic acid ester does not rigidify by drying but by flocculation in the second solvent in which it is insoluble. Therefore the ester remains voluminous distributed in the pore interstices and results in thick pore walls with foam-like ultrastructure.

Applicants further note that each of the cited documents discloses a solution resulting in a specific matrix or gel. However, there is no motivation for skilled artisan to pick one component out of one document and to transfer it to the teaching of another document. For this additional reason as well, a *prima facie* case of obviousness does not exist. Withdrawal of the rejections, is therefore, solicited. Applicants intend to submit a declaration regarding the points enumerated above

CONCLUSION

In view of the instant amendments and remarks, reconsideration and allowance of all pending claims is requested. If there are any issues remaining that the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the local exchange listed below.

Respectfully submitted,

Date: July 14, 2003

Marvin Motsenbocker

Marvin Motsenbocker
Reg. No.: 36,614

Heller Ehrman White & McAuliffe LLP
1666 K Street, N.W., Suite 300
Washington, D.C. 20006-4004
Telephone: (202) 912-2000
Facsimile: (202) 912-2020